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A Study on *in-vitro* Application of N P K Fertilizers to Control Haemonchosis in Grazing Animals

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ABSTRACT

Possibility of in-vitro use of N P K fertilizers to control haemonchosis has been evaluated in grazing animals. For this *Haemonchus contortus eggs* were collected from adult *H. contortus* parasite. For in-vitro study egg hatch assay was done. The different concentrations (g per cent) of N, P, K fertilizers were used as 0, 0.25, 0.50, 1.00, 2.00 and 4.00 g per cent. Commercial fertilizers like urea (N-46%), Single Super Phosphate (SSP) (P-16%) and Muriate Of Potash (MOP) (K- 60%) were used. Total 160 number of micro centrifuge tubes of 1.5 ml were taken and in those tubes solution of urea, SSP, MOP (400 µl) were taken and to them 100 µl of egg suspension containing about 200 eggs of *H. contortus* were added. In control, only water was added. The tubes were incubated. Then the contents of each tube were counted under microscope to calculate egg hatching. Results revealed that among N, P, K fertilizers P was most lethal for *H. contortus* eggs. P fertilizer at concentration of 1 g per cent or more, completely inhibited egg hatching of *H. contortus* parasite. K fertilizer totally blocked the egg hatching at 4 g per cent or more concentration.

Key words *Haemonchus contortus, In-vitro, N P K fertilizer*

Many times parasitism causes heavy economic loss to the profitable livestock farming in form of morbidity as well as mortality. Although it is less evident in stall fed animals, but very large number of domesticated pasture grazing animals become the victim of parasitic infections. Gastrointestinal parasitic infection such as fasciolosis, paramphistomosis, strongyle infection are mostly found in tropical countries like India. The abomasal nematode parasite, *Haemonchus contortus* contributes the major share of all those due to its wide distribution and high pathogenicity in ruminants. It causes huge production loss resulting in anemia, anorexia, depression, loss of condition and eventual death of animals. Pasture plays an important role for completion of different developmental stages of life cycle of the parasite acting as a potential source of infection. So the habit of pasture grazing in domestic animals compels them to fall victim of haemonchosis.

Though organic farming is advocated today, but widely N P K fertilizers are applied in crops, pasture land and agricultural operations for fortifying the soil. Along with the property of enriching the soil for high production, these fertilizers had reported to be detrimental effect on hatching and development of larvae of animal parasites. So the present study has been envisaged on in-vitro application of N P K fertilizers in form of urea (N- 46%), single super phosphate (SSP) (P-16%) and muriate of potash (MOP) (K- 60%) to control haemonchosis in grazing animals.

MATERIALS AND METHODS

There was no use of live animals in the experiment and it was strictly in accordance with the institutional ethical committee guidelines. The present study was carried out for a period of 1 year inside Department of Veterinary Parasitology and other Departments of College of Veterinary Science and Animal Husbandry, Bhubaneswar, Odisha. Adult *H. contortus* parasites were collected and recovered, distinguished from their barber's pole appearance and other morphological features (Soulsby, 1982) from gastrointestinal tracts (abomasum) of goats slaughtered at local abattoirs of Bhubaneswar, Odisha. Fertilized *H. contortus* eggs were collected from them by dissecting the worms in PBS (Khatun *et al*., 2013). Eggs were counted per ml of suspension in PBS and stored at 15 ^{0}C for further use.

For in-vitro study egg hatch assay was done. The different concentrations (gram percentage, g percent) of N, P, K fertilizers were varied as 0 g percent (control), 0.25 g per cent, 0.50 g percent, 1.00 g percent, 2.00 g percent, and 4.00 g percent). Commercial fertilizers like urea (N- 46%), SSP (P-16%) and MOP (K- 60%) were used. Basing on the above (46 g of N is present in 100 g of urea, 7 g of P is present in 100 g of SSP, 50 g of K is present in 100 g of MOP), the quantity of g percent (0.25, 0.50, 1.00, 2.00, 4.00) of N, P, K were calculated and represented (table-1). Total 160 number of micro centrifuge tubes of 1.5 ml were used (10 replicates of control, 10 numbers of tubes for each concentration of N, P, K). In those tubes solution of urea, SSP, MOP (400 µl) were taken and to them 100 µl of egg suspension containing about 200 eggs of *H. contortus* (Swarnkar *et al*., 2013) were added. In control, only water was added. The tubes were kept open and incubated at 27 ^{0}C for 24 hours. Then the contents of each tube were counted under microscope for hatched larvae and dead eggs to calculate egg hatching rate (%) by putting a drop of Lugol's iodine.

Statistical analysis

The percentage data from egg hatch assay were subjected to arc-transformation and analyzed for significance by ANOVA. The means were compared by DMRT using SPSS programme version 16.

RESULTS AND DISCUSSION

The mean egg hatching percentage in control and N, P, K, fertilizers irrespective of different concentrations (g percent) were found 84.6±0.26, 47.00±0.11,7.5±0.11 and 28.4±0.11 respectively, which differed significantly ($p<0.05$) from each other. So it was noticed that the egg hatching per

Table 1. Requirement of N (urea), P (SSP), K (MOP) in form of g percent

Treatments	Nitrogen (N) g% / phosphorus (P) g% / potassium (K) g%	Urea in g	SSP in g	MOP in g	Water (ml)
T-1	0.00	0.00	0.00	0.00	100
T-2	0.25	0.543	3.57	0.50	100
T-3	0.50	1.09	7.15	1.00	100
T-4	1.00	2.17	14.29	2.00	100
T-5	2.00	4.34	28.58	4.00	100
T-6	4.00	8.68	57.16	8.00	100

cent was highest in N and lowest in P fertilizer. This is in accordance with Dakul, *et al*., 2000, who showed that SSP was most ideal for causing mortality of *Schistosoma mansoni* cercariae in laboratory condition as compared to urea.

The mean egg hatching per cent in control and fertilizers N, P, K individually with respect to different concentration (g per cent) were represented (Table-2).

Egg hatching in each concentration of N fertilizer differed significantly ($p>0.05$) from 81.6±0.26 to 2.3±0.26. The dose rate 0.25 g per cent of N fertilizer showed highest hatchability (81.6±0.26%). Gradually due to increased dose the potency might be enhanced which caused decreasing hatchability. Urea could not prevent development of eggs to larvae at 0.25 to 4.00 g per cent. Our result is in agreement with JeyaThilakan and Sathianesan, 2007, reported that urea was an efficient ovicidal agent for nematode eggs at 5 % concentrations and not below that.

Egg hatching in 0, 0.25, 0.50 g per cent concentrations of P fertilizer differed significantly ($p<0.05$) from each other but 100 per cent mortality was seen in 1.00, 2.00 and 4.00 g per cent concentration (no significant difference, $p>0.05$). In 0.25 g per cent concentration of P fertilizer the hatchability was 59.3±0.26%. By increasing the dose hatching per cent was gradually decreased. At 1, 2, 4 g per cent of P the hatchability was reduced to zero. So with 1 g per cent or more than that, P was found to be detrimental for egg development. Reduced development might be due to high potency of P fertilizer at higher concentrations.

In case of K fertilizer, the mean egg hatching per cent in different g per cent concentration varied from 70.1±0.26 to 0±0.26. For K fertilizer in 0.25 g per cent, the hatching rate was noticed to be 70.1±0.26%. By the gradual increase of concentration the hatching was decreasing. There was 12.6±0.26 % hatching found in 2.00 g per cent of K fertilizer. When the concentration became 4 g per cent the hatchability was completely blocked (0%). The reason could be higher potency at higher doses. Sommerville, 1976, reported that at or below 0.015 mM of potassium ion development of infective larva was almost completely stopped.Significant difference ($p<0.05$) were seen among 0.25, 0.50, 1.00, 2.00 g per cent concentrations with all these fertilizers. Significant difference ($p<0.05$) in case of 4 g per cent in between N and either P or K fertilizer were found. There were no significant difference ($p>0.05$) noticed in between 0.50 g percent of P and 2.00 g per cent of N.

Urea was found to be fatal for earthworm of *Eisenia foetida*, through skin infiltration (Rai *et al*., 2014) and also for filarial vector mosquito, *Culex pipiens pipiens* (Olayemi *et al*., 2012). Some researchers found that inorganic fertilizer (NPK) caused suppression of population of cereal cyst nematode, *Heterodera avenae* population (Al-Hazmi *et al*., 2014) as well as root-knot nematode population (Abolusoro *et al*., 2013, Karerajeh and Farah, 2014, Nehal and Abdl-Kader, 2014, Ramezani and Khaniki, 2015, Shashraf *et al*., 2014). Phosphorus fertilizers acted as nematicidal for species of root knot nematodes, *Meloidogyne javanica* and *M. incognita*due to their low pH value, salinity and decline in lipid content (Habash and Luma, 2011). The delayed or blocked hatching of *H. contortus* eggs by fertilizer action might be as a result of disturbance in the secretion or loss of function of certain enzymes present in the eggs responsible for the hatching process (Munir *et al*., 2001).

Table 2. Effect of N, P, K fertilizers in different concentrations on egg hatching

Concentrations of fertilizers (g per cent)	Hatching per cent in N fertilizer	Hatching per cent in P fertilizer	Hatching per cent in K fertilizer
0	84.6^{a}	84.6^{a}	84.6^{a}
0.25	81.6^{b}	59.3^{f}	70.1^{d}
0.50	78.4^{c}	23.7^{i}	52.6^{g}
1.00	61.3^{e}	0^{l}	36.2^{h}
2.00	23.6^{i}	0^{l}	12.6^{j}
4.00	2.3^{k}	0^{l}	0^{l}
	SEM 0.26	SEM 0.26	SEM 0.26

Means with different superscripts differ significantly ($p<0.05$) from each other in a row or a column

Furthermore, by release of free radicals (Sarma *et al*., 2010) as well as various toxic metabolites from fertilizers, it hindered the hatching of parasitic eggs and subsequent development.Urea, potash, NPK fertilizer badly affected the hatching and larval development of *Aedes aegypti* mosquito by disturbance of pH, conductivity and total dissolved solid of egg and larva (Darriet, 2016, Kumar *et al*., 2015).

So among N, P, K fertilizers P was found to be the most effective one in preventing the hatching of *H. contortus* eggs than N and K fertilizer. P fertilizer at concentration of 1 g per cent or more, completely inhibited egg hatching of *H. contortus* parasite. K fertilizer totally blocked the egg hatching at 4 g per cent or more concentration. Our study concluded that, application of N-P-K fertilizers at recommended doses in pasture land can inhibit larval translation of *Haemonchus contortus* in grazing animals.

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